Elucidation of chemical pathways in the Maillard reaction by $^{17}$O NMR Spectroscopy

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What does mechanism mean?

- A mechanism is the **actual** process by which a reaction takes place, which bonds are broken, in what order, how many steps are involved, the relative rate of each step, etc, …

- The usual course is that the gross features of a mechanism are the first to be known and then increasing attention is paid to finer details.

- In some cases there are several proposed mechanisms, each of which explains all the data.

- One proposal is right up to the contrary is demonstrated.

(Jerry March)
Simplified scheme of the Initial phase of the Maillard Reaction
Objective: Study the formation of short-chain organic acids
Formation of acetic and formic acid from deoxyosones

- Synthesis of 1-deoxy-2,3-hexodiulose (1-Deoxyglucosone, 1-DG)

\[
\begin{align*}
\text{Reagents and conditions:} & \quad \text{a) ethoxyvinyllithium (40\%)} \\
& \quad \text{b) HCl 1N (78 \%)} \\
& \quad \text{c) DOWEX 50X8 (26 \%)}
\end{align*}
\]

- 3-Deoxyhexo-2-ulose (3-Deoxyglucosone, 3-DG) is commercially available
Application of $^{17}$O NMR spectroscopy to the Maillard reaction

- **Advantages**
  - Allows to follow specific incorporation in the whole mixture
  - Leads to easy to read spectrum

- **Possible limitations**
  - Poor resolution
  - Time-consuming experiments ($^{17}$O is only 0.037 %)
  - Price of H2$^{17}$O enriched water

- **Experimental conditions:**
  - Spectral width: 100 kHz
  - 90°C pulse angle 9.9 $\mu$s
  - 200 ms acquisition delay
  - 41 ms acquisition time
  - Pulse sequence: P1 (90°C), P2 (90°C), P3 (90°C)
  - 20 Hz exponential broadening factor to the FID applied before FT
1-DG as substrate

- One main signal corresponding to acetic acid (checked by spiking)
- A broader signal, possibly erythronic acid
3-DG as substrate

• One main signal corresponding to formic acid (checked by spiking)
• A broader signal, possibly an organic acid
Glucose as substrate

- Two signals corresponding to acetic and formic acid
- Two other signals, probably organic acid as well
To summarize

- Labeled water provides clean NMR spectrum evidencing the incorporation of oxygen from water in Maillard reaction products.
- Acetic and formic acids formed are at least partially labeled.
- No link can be establish about quantification.
What is the role of oxygen?

Glycine, $\text{H}_2\text{O}, ^{18}\text{O}_2$, 90°C, pH 8, phosphate buffer

Acid acetic formation

Conversion: 32%
Labeled: 30%
Experiment with $^{18}$O-labeled water and oxygen

Glycine, $\text{H}_2^{18}\text{O}$, $^{18}\text{O}_2$, 90°C, pH 6, no buffer

Acid acetic formation

- Unlabeled and mono-labeled acetic acid was found (m/z 60/62)
- Thus, there is incorporation of only one labeled oxygen atom
Possible Mechanism proposed

\[ \text{SET} \quad \text{Ox.} \quad \text{B.V.R.} \quad \text{SET} \]

\[ \text{HO} \quad \text{CO}_2\text{H} \quad \text{HO} \quad \text{CO}_2\text{H} \]

\[ \text{OH} \quad \text{OH} \quad \text{OH} \quad \text{OH} \]

\[ \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \]

\[ \text{O}^* \quad \text{O}^* \quad \text{O}^* \quad \text{O}^* \]

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\[ \text{OH} \quad \text{OH} \quad \text{OH} \quad \text{OH} \]

\[ \text{CO}_2\text{H} \quad \text{CO}_2\text{H} \quad \text{CO}_2\text{H} \quad \text{CO}_2\text{H} \]

\[ \text{OH} \quad \text{OH} \quad \text{OH} \quad \text{OH} \]

\[ \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \]
Experiment without using labeled compounds

Glycine, H₂O, Ar, 90°C, pH 8, phosphate buffer

conversion: 32%

- Oxygen from O₂ can be incorporated, but is not strictly necessary
- Thus, there must be several mechanisms leading to acetic acid

Sugar → Acetic acid

Reactivity always follows the easiest way!
Conclusions

• Formation of acids from sugars and α-dicarbonyls may proceed via a Baeyer-Villiger rearrangement

• Oxygen incorporated in the acids may originate from molecular oxygen or water

• $^{17}$O NMR spectroscopy in combination with targeted labeling experiments provide new insight in formation mechanisms in the Maillard reaction